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(57) Abstract: The present invention provides a method for determining the dosage of FSH in the treatment of infertility of women comprising the determination of the FSH receptor variant of the soman to be treated, a method for treating infertility in women which comprises said determination of the FSH receptor variant and a kit for performing said determination of the FSH receptor variant.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEIKOOP J C ET AL: "Infertility treatment by recombinant FSH" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 61, no. 3, 13 May 1998 (1998-05-13), pages 233-236, XP004128060 ISSN: 0168-1656 the whole document	11
Y	---	1-5, 7, 9, 10
Y	BEN-RAFAEL Z ET AL.: "Pharmacokinetics of follicle-stimulating hormone: clinical significance" FERTILITY AND STERILITY, vol. 69, no. 3Sp2, March 1998 (1998-03), pages 40S-49S, XP001023707 the whole document	1-5, 7, 9, 10
	----	-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
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- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

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PCT/EP 00/12886	

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CONWAY G.S. ET AL.: "Mutation screening and isoform prevalence of the follicle stimulating hormone receptor gene in women with premature ovarian failure, resistant ovary syndrome and polycystic ovary syndrome" CLINICAL ENDOCRINOLOGY, vol. 51, July 1999 (1999-07), pages 97-99, XP000920627 cited in the application the whole document ---	1-5,7,9
Y	SIMONI M. ET AL.: "Mutational analysis of the follicle-stimulating hormone (FSH) receptor in normal and infertile men: identification and characterization of two discrete FSH receptor isoforms" JOURNAL OF CLIN. ENDOCRINOLOGY AND METABOLISM, vol. 84, no. 2, - February 1999 (1999-02) pages 751-755, XP000920695 cited in the application * see especially page 752, column 1, paragraphs 3-4 * the whole document ---	1-5,9,10
Y	WO 97 11194 A (UNIV HELSINKI LICENSING ;CHAPELLE ALBERT DE (FI); AITTOMAEKI KRIST) 27 March 1997 (1997-03-27) the whole document ---	1-5,9,10
A	JIANG M ET AL.: "The frequency of a inactivating point mutation (566C-T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry" JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, vol. 83, no. 12, 1998, pages 4338-4343, XP001023798 the whole document ---	
A	LAYMAN L C ET AL.: "The Finnish follicle-stimulating hormone receptor gene mutation is rare in North American women with 46,XX ovarian failure" FERTILITY AND STERILITY, vol. 69, no. 2, 1998, pages 300-302, XP001023705 the whole document ---	
		-/-

INTERNATIONAL SEARCH REPORT

Item: 1 Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GROMOLL J ET AL: "AN ACTIVATING MUTATION OF THE FOLLICLE-STIMULATING HORMONE RECEPTOR AUTONAMOUSLY SUSTAINS SPERMATOGENESIS IN A HYPOPHYSECTOMIZED MAN"</p> <p>JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, NEW YORK, NY, US, vol. 81, no. 4, 1 April 1996 (1996-04-01), pages 1367-1370, XP000609740 ISSN: 0021-972X the whole document</p> <p>---</p>	
A	<p>WO 98 34948 A (CORNELL RES FOUNDATION INC) 13 August 1998 (1998-08-13) * see especially SEQ ID NO:25 * abstract</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 9711194 A	27-03-1997	US 5851768 A AU 6990296 A		22-12-1998 09-04-1997
WO 9834948 A	13-08-1998	AU 6149898 A EP 1015478 A		26-08-1998 05-07-2000

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(57) Abstract: The present invention provides a method for determining the dosage of FSH in the treatment of infertility of women comprising the determination of the FSH receptor variant of the soman to be treated, a method for treating infertility in women which comprises said determination of the FSH receptor variant and a kit for performing said determination of the FSH receptor variant.

**Genetic variants of the human FSH receptor and the influence thereof on
gametogenesis**

5

The present invention provides a method for determining the dosage of FSH in the treatment of infertility of women comprising the determination of the FSH receptor variant of the woman to be treated, a method for treating infertility in women which comprises said determination of the FSH receptor variant and a kit for performing 10 said determination of the FSH receptor variant.

The success of controlled ovulation induction in the course of an assisted reproduction procedure depends on the administration of the hormone FSH. Unfortunately neither the patients's reaction towards the administration of FSH nor which hormone 15 dose is necessary can be foreseen at the beginning of the treatment phase. Due to the lack of predictive parameters a high number of expensive ampoules of FSH is being used in IVF clinics for the induction of ovulation, a treatment regimen that bears the danger of overstimulation and its clinical consequences.

20 The follicle-stimulating hormone (FSH) is an essential factor for the maturation of germ cells (gametogenesis) in both men and women. FSH exerts its action via the FSH receptor which is specifically located in the granulosa cells in the ovary and in the Sertoli cells in the testis. Any perturbation of the interaction between FSH and its receptor leads to impairment of gametogenesis. Women with FSH receptor mutations 25 show a clinical picture typical of primary amenorrhoea, men with FSH receptor mutation are generally subfertile. These observations underline the central role of FSH and its receptor for a normal physiological maturation of oocytes as well as spermatozoa (Nieschlag et al., Clin. Endocrinol. 51:139-146 (1999)).

30 The FSH receptor is present on the cell membrane and consists of an extracellular, a transmembrane and an intracellular domain. The FSH receptor gene is located on chromosome 2p21 and consists of 10 exons. Exons 1 to 9 encode for the

extracellular domain while exon 10 encodes for the transmembrane and intracellular domain. The whole gene spans a region of over 54 kbp encoding a mature protein of 695 amino acids (Gromoll et al. Genomics 35, 308-311 (1996)).

5 Our recent studies have shown that the FSH receptor exists in two genetic variants. Amino acid position 307 is either occupied by alanine or threonine and at position 680 either serine or asparagine is found. The amino acid position 307 is located in the extracellular domain while the amino acids at position 680 are part of the intra-cellular domain. We have shown that both positions are genetically linked with each
10 other. Due to that two discrete FSH receptor variants can be usually found consisting of either threonine 307 and asparagine 680 or alanine 307 and serine 680 (Fig. 1). Both receptor variants are statistically equally distributed. Further, Simoni et al., J. Clin. Endocrinol. Metab. 84:751-755 (1999) and Conway et al., Clinical Endocrinology, vol. 51, 97-99 (1999) describes that the FSH receptor variants can be analysed
15 by restriction enzyme analysis.

So far functional studies could not show any significant differences as to hormone binding or signal transduction between the two receptor variants. It should be noted, however, that the model system used so far for functional studies is not sensitive
20 enough to detect subtle differences in FSH-FSH receptor interaction and receptor activation. In a first clinical study comparing infertile men with fertile men no significant differences of receptor variant distribution could be detected (Simoni et al., J. Clin. Endocrinol. Metab. 84:751-755 (1999)).

25 In an additional study completed recently we have investigated normal women who were treated with FSH in the course of an assisted reproduction procedure. The administration of FSH leads to a controlled induction of ovulation which makes it possible to gain oocytes from the patient. The oocytes are then incubated with sperm in vitro and the nascent zygotes are re-implanted into the patient's uterus. The aim of
30 the FSH treatment is to gain a sufficient number of oocytes capable of being fertilized. Clinical experience shows, however, that patients react differently towards the stimulation with FSH. While some patients need relatively low doses of FSH in order

to produce a sufficient number of oocytes, other patients have to be stimulated with high doses of FSH to reach the same results in terms of a sufficient number of oocytes. Depending on the amount of FSH necessary the patients can be classified into good responders (low dose of FSH necessary) and bad responders (high dose of FSH necessary). The reasons for the difference in sensitivity towards stimulation with FSH are so far not known. The need for adjusting the FSH dose over the course of time during the stimulation phase in order to give rise to a sufficient number of oocytes without provoking an overstimulation represents a major problem in IVF treatment. The clinical picture of differences in the sensitivity towards FSH in patients undergoing assisted reproduction procedure was the starting point for our investigation in which we tested the hypothesis that different FSH receptor variants are responsible for the differences in FSH sensitivity.

We have screened 160 patients from our IVF department and could show that patients bearing the homozygous FSH receptor variant alanine 307/serine 680 need significantly more FSH for the stimulation of oocyte maturation than the patients with the homozygous FSH receptor variant threonine 307/asparagine 680. It became further obvious that in patients with a heterozygous state of FSH receptor variants an intermediate dose of FSH was necessary for ovulation induction (Fig. 2). Moreover, the receptor variants seem to regulate the basal serum levels of FSH since patients bearing the homozygous FSH receptor variant alanine 307/serine 680 show a mean basal FSH level of 7.9 IU/l, while in patients with a heterozygous receptor variant and in patients homozygous for the FSH receptor variant threonine 307/asparagine 680 the mean basal FSH level is 7.0 IU/l and 6.3 IU/l, respectively (Fig. 3).

25

Thus, it was found that the response of patients towards the stimulation with FSH is depending on the allelic variant of the FSH receptor. In order to determine which particular variant of the FSH receptor is present in a given patient a simple analysis of DNA extracted from blood cells has to be conducted. The individual amount of FSH to be administered could be determined according to which FSH receptor variant the patient possesses. Due to these findings it can be expected that in the future the genetic analysis of the FSH receptor region determining the variant will play an

important role for the planning of ovulation induction treatment. In other words, it was found that the FSH receptor variants determine differences in sensitivity towards FSH. This finding has a great impact on the FSH therapy in the course of an assisted reproduction procedure since it makes a predetermined and individually adjusted

5 FSH stimulation protocol possible depending on the FSH receptor variant present in a given patient. The potential benefits of such an adjusted FSH stimulation therapy are profound. This approach will not only improve the clinical safety, by avoiding dangerous overstimulation with FSH, but will also help to reduce treatment costs significantly, since FSH is quite expensive.

10

Finally, FSH receptor variants may be associated with reduced fertility in men and women and that the analysis of such variants may have great impact on the treatment of reduced fertility with FSH.

15 The invention thus provides

(1) a method for determining, i.e., predicting the dosage of follicle-stimulating hormone (FSH) in the treatment of infertility of women comprising the determination of the FSH receptor variant of the woman to be treated;

20 (2) a method for treating infertility in women which comprises determining the FSH receptor variant in the woman to be treated as defined in (1) above;

(3) a kit for performing the determination of the FSH receptor variant in a woman as defined in (1) and (2) above; and

(4) a FSH preparation comprising a specific amount of FSH which is suitable as a daily dosage for high dosage, intermediate dosage or low dosage FSH treatment.

25

The attached Figures 1 to 6 depict the following:

Fig. 1 shows the two FSH receptor variants.

30 Fig. 2 shows the number of FSH ampoules (75 IU/ampoule) required to achieve ovulation induction and oocyte retrieval in normoovulatory women in an assisted reproduction programme.

(data: mean ± SEM)

* p < 0.05 vs A/A (Kruskall Wallis test)

Fig. 3 shows the basal FSH levels (day 3) in normoovulatory women grouped according to the FSH receptor genotype.

(data: mean ± SEM)

*p < 0.05 vs A/A (Kruskall Wallis test)

Fig. 4 shows the restriction fragment length polymorphism of Asn 680 Ser of the FSH receptor.

Fig. 5 shows the DNA sequence of exon 10 of the FSH receptor gene (EMBL accession No. X91747).

Fig. 6 shows exon 10 of the FSH receptor. Suitable primers are underlined. The reverse primers A₂-G₂ are complementary to the respective underlined sequences in this figure.

The method (1) of the invention is hereinafter described in more detail. First of all, the determination of the FSH receptor variant is preferably performed *in vitro*.

Secondly, since the polymorphic sites are genetically coupled in the FSH receptor variants, the sequence analysis of one variant site is sufficient. It is preferred that the analysis is performed by the "restriction fragment length polymorphism" (RFLP) method. The first step of said method is the isolation of genomic DNA from a patient's blood sample and the amplification of a specific part of the FSH receptor by PCR. The amplicon obtained in the PCR is cut with a restriction enzyme which specifically recognizes the amino acid sequence at position 680, e.g. Bsr I. A complete restriction of the amplicon indicates the presence of a homozygous serine, in the case of an incomplete restriction a heterozygous receptor status is present and no restriction of the amplicon indicates a homozygous asparagine (Fig. 4). Suitable primers for the PCR reaction are shown in Fig. 6.

The determination of the variant may also be performed by the "single stranded conformation polymorphism" (SSCP) method and/or by the "allele specific amplification" method. The RFLP, SSCP and "allele specific amplification" methods are generally known in the art (e.g. from Oldenburg, M.C. and Siebert, M., New Cleavage Fragment Length Polymorphism method improves the mutation detection assay, *BioTechniques*, Feb. (2000), 28(2):351 and Shi, M. M. et al., Technologies for detecting genetic polymorphisms in pharmacogenomics, *Mol. Diagn.* Dec. (1999), 4(4):343-51), which we hereby incorporate by reference.

10

The detection of a single nucleotide change leading to an amino acid exchange is ideally suited for the development of a specific kit which would make the detection of the FSH receptor variants easy and fast. Such a kit would ideally be used for the screening of patients prior to a FSH therapy.

15

In the FSH therapy and after determination of the FSH receptor, the women bearing the homozygous FSH receptor variant Ala307/Ser680 may be given a high dosage of FSH, namely about 42-48 ampoules FSH within a treatment period of 14 days, which corresponds to a daily dosage of greater than 225, preferably 230 to 250 International Units (IU) FSH; the women bearing the homozygous FSH receptor variant Thr307/Asn680 may be given a low dosage of FSH, namely 30-35 ampoules FSH per 14 days, which corresponds to a daily dosage of 150 ± 20 IU FSH; and the women with a heterozygous state may be given an intermediate dosage of FSH, namely about 36 to 41 ampoules per day, which corresponds to a daily dosage of 200 ± 20 IU FSH. The FSH is preferably administered subcutaneously.

The FSH preparation of embodiment (4) of the invention contains the high dosage, intermediate dosage or a low dosage FSH as set forth in detail above. The preparation is preferably in an injectable form and may contain suitable additives (such as buffers, saline, etc.) known in the art.

The invention is further illustrated by the following non-limitative example.

Example 1: Determination of the restriction fragment length polymorphism (Asn680Ser, hFSH receptor)

5

A. PCR: Amplification with primers E₁ and G₂ (Exon 10 of the FSH receptor)

For each sample is pipetted:

36 µl autoclaved distilled water

10 5 µl Thermo-Buffer 10x (Promega)

3 µl MgCl₂ (25 mM, Promega)

2.5 µl dNTP-solution (1mM, Pharmacia)

1 µl Primer E₁ (0.1 µg/µl; 5'-CCTTGTGCTAATGTCCTGG)

1 µl Primer G₂ (0.1 µg/µl; 5'-TGTAGAACGCACTGTCAGCTC)

15 0.5 µl Taq DNA polymerase (5000 IU/ml, Promega)

1 µl DNA (DNA extracted from 5-10 ml and dissolved in 50 µl distilled water)

PCR program:

94°C 4 min 1 cycle

20

94°C 1 min

58°C 30 s 35 cycles

72°C 50 s

25 72°C 10 min 1 cycle

30°C 30 min

The PCR product is checked on a 2% TAE agarose gel. The size of the desired band is 580 kbp.

30 Subsequently a phenol-chloroform cleaning is performed twice, the resulting DNA is precipitated with 0.5 sample volumes 7.5 M ammonium acetate and 2.5 volumes ab-

solute ethanol, washed with 70% ethanol and air-dried. Finally, the DNA is re-dissolved in 17 µl water.

B. Digestion:

5

2 µl buffer NEB 3 10x (Biolabs) and

1 µl Bsr I (Biolabs) are added to the sample, the resulting mixture is overlayed with 2 drops of mineral oil and digested for 1.5 hours at 65°C. The digestion is checked on a 2-2.5% TAE agarose gel.

10

The enzyme Bsr I has a restriction site for TGACC. If the FSH receptor contains the amino acid serin at position 680 of the 5th transmembrane domain, Bsr I cuts the PCR product in two bands (443 and 136 bp). The enzyme cannot digest the PCR product if the amino acid at position 680 is asparagin. The single band on the gel has a size of 579 bp (see Fig. 4).

15

C. Results:

One band size 579 bp	→	asparagin, homozygous
Two band sizes 443 and 136 bp	→	serin, homozygous
Three band sizes 579, 443 and 136 bp	→	asparagin/serin heterozygous

20

Example 2: We started a prospective study which women were screened for the FSH receptor variant before starting the FSH treatment. Only women with homozygous

25

FSH receptor variant at position 680 were included in the study and were randomised to receive a pre-determined, fixed dosage of FSH. The preliminary results, based on 32 cycles, confirm that the Ser 680 variant is less sensitive to FSH stimulation (in terms of production of estradiol) and that more FSH is necessary to induce the degree of stimulation observed in women with the Asn 680 variant.

30

These results reinforce the idea that the analysis of the FSH receptor variant is useful for the determination of the FSH dosage.

CLAIMS:

1. Method for determining the dosage of follicle-stimulating hormone (FSH) in the treatment of infertility of women comprising the determination of the FSH receptor variant of the woman to be treated.
2. The method of claim 1, wherein the determination of the FSH receptor variant comprises the steps:
 - 10 (a) isolating genomic DNA from a blood sample of the woman to be treated, and
 - (b) determining whether the isolated DNA codes for the FSH-receptor variant Ala 307/Ser 680 or Thr 307/Asn 680.
3. The method of claim 2, wherein the determination of the FSH-receptor variant of step (b) is performed by
 - (b1) partial amplification of the FSH receptor DNA by use of a pair of primers flanking the variant region(s) of the FSH receptor DNA coding for the amino acids 307 and/or 680 of the FSH receptor protein,
 - (b2) digesting the amplified DNA with a restriction enzyme digesting only the DNA of one of the FSH receptor variants,
 - (b3) determining the FSH-receptor variant by restriction fragment length-polymerase chain reaction.
4. The method of claim 3, wherein the length of the primers is 12 to 30 nucleotides, preferably 17 to 25 nucleotides, and the distance to the nucleotides coding for the amino acid in positions 307 or 680 are 20 to 1500 bp, preferably 100 to 1000 bp.
5. The method of claim 3 or 4, wherein the primers are flanking the DNA sequence of the amino acid in position 680 of the FSH receptor protein and the restriction enzyme is Bsr I.

6. The method of claims 3 to 5, wherein the pair of primers comprises an upstream primer selected from

A₁: 5'-GCTATACTGGATCTGAGATG

B₁: 5'-TTGACATGACGTACACTGAG

5 C₁: 5'-CTGATCTCTGCATTGGAATC

D₁: 5'-AGCTGGACTGCAAGGTGCAG

E₁: 5'-CCTTGTGCTCAATGTCCCTGG

F₁: 5'-CCATTCTGCCTCCCTCAAG

G₁: 5'-GAGCAAGTGTGGCTGCTATG,

10 and a reverse primer selected from

A₂: 5'-ACCACTTCATTGCATAAGTC

B₂: 5'-CAACTGATGCAATGAGCAGC

C₂: 5'-ATCCAGCCCATCACCATGAC

D₂: 5'-GGTTCCGCACTGTGAGGTAG

15 E₂: 5'-GCTTGACACAGTGATGAG

F₂: 5'-TGGATGGGTGTTGTGGACAG

G₂: 5'-TGTAGAACGACTGTCAGCTC,

preferably the pair of primers is E₁ and G₂ as defined above.

20 7. A method for treating infertility in women which comprises determining the FSH receptor variant in the woman to be treated as defined in claims 1 to 6.

8. The method of claim 7, further comprising administering the woman a suitable amount of FSH.

25

9. A kit for performing the determination of the FSH receptor variant in a woman as defined in claims 1 to 8.

30 10. The kit of claim 9 comprising a pair of primers as defined in claims 3-6, Taq polymerase and a restriction enzyme.

11. A FSH preparation comprising a specific amount of FSH which is suitable as a daily dosage for high dosage, intermediate dosage or low dosage FSH treatment.

Fig. 1

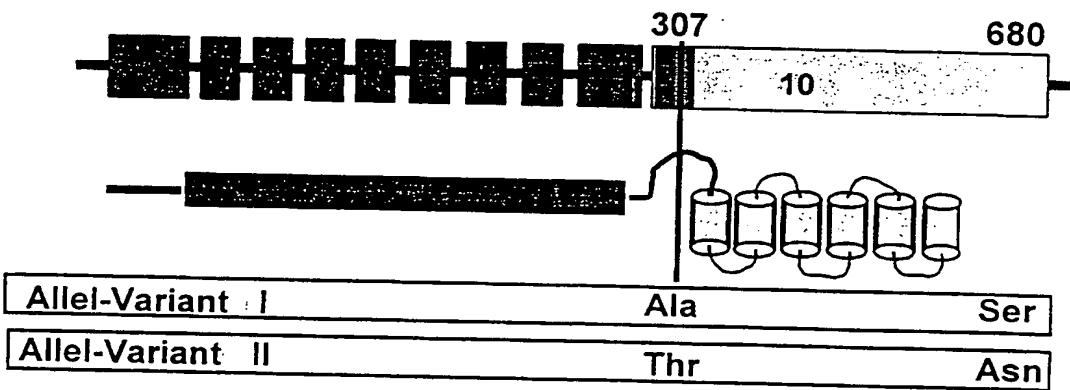


Fig. 2

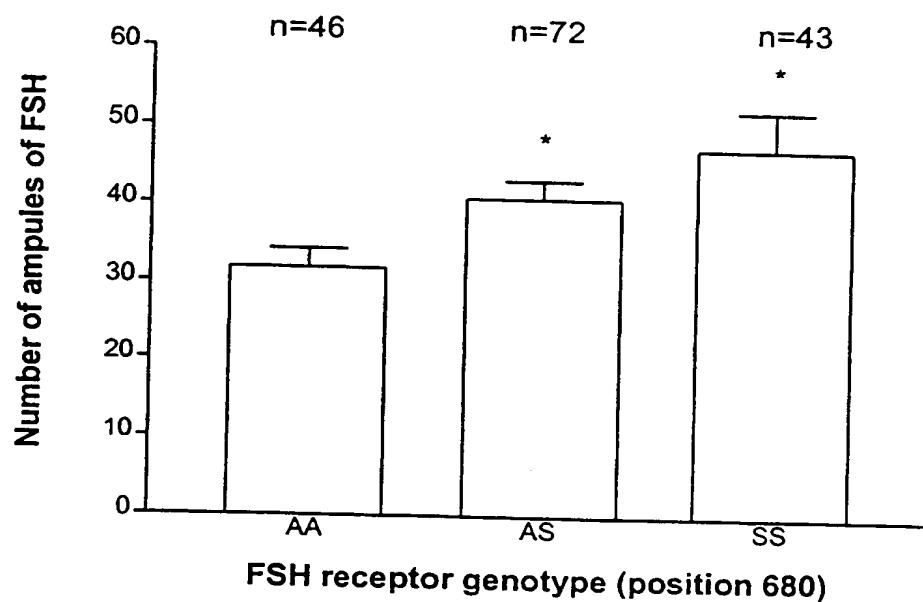


Fig. 3

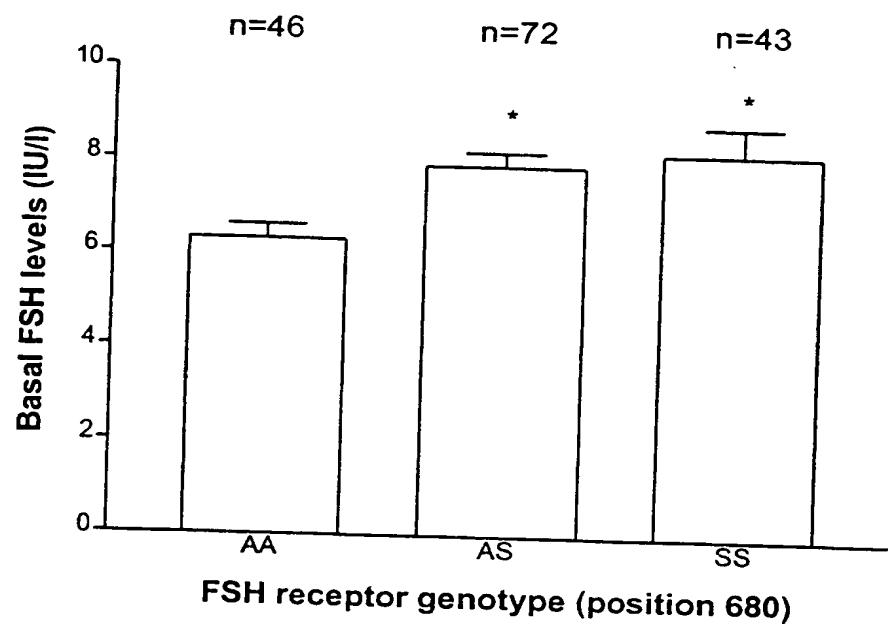


Fig. 4

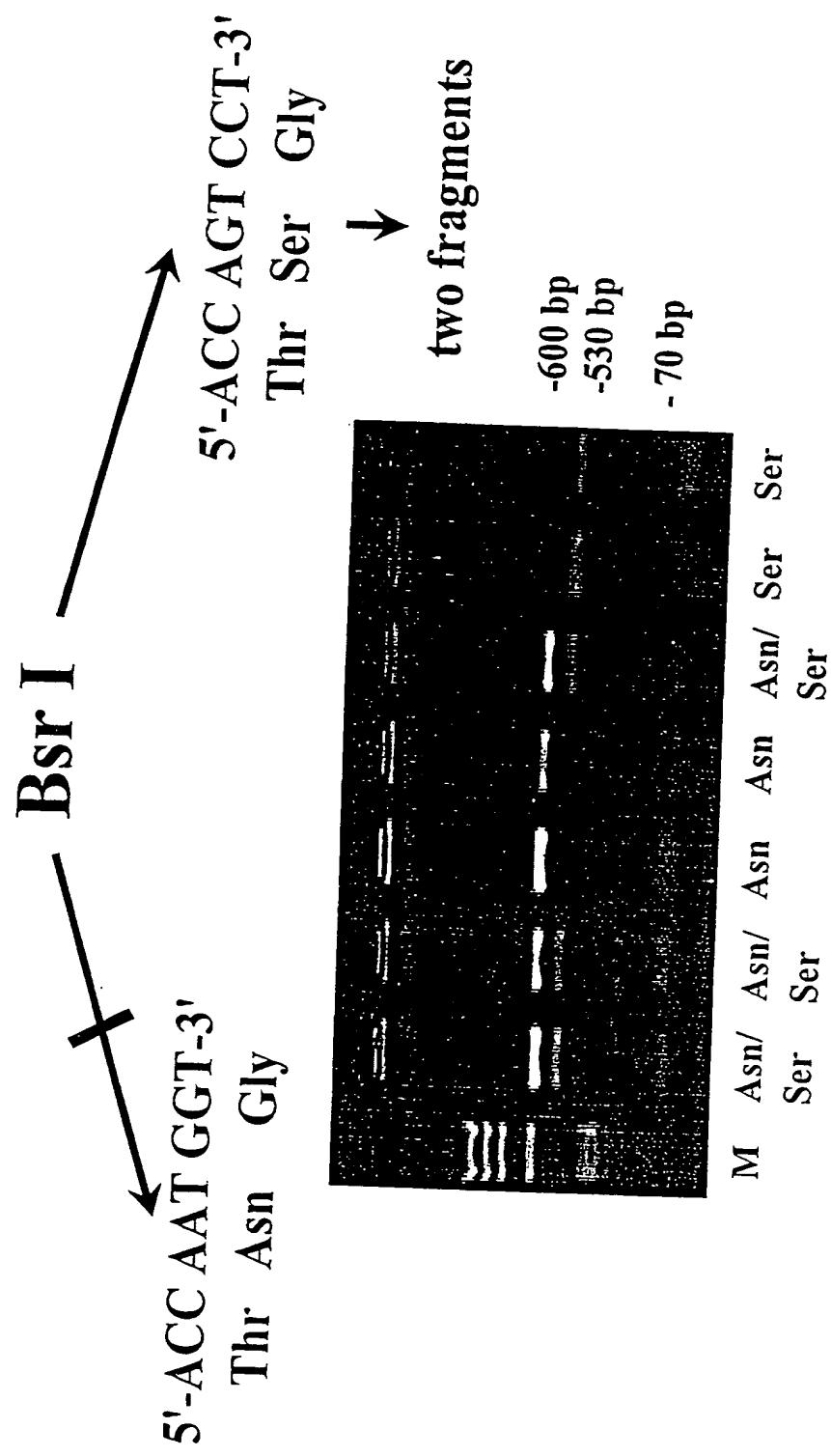


Fig. 5

tctagctctg agcttcatcc aatttgcaac aaatctattt taaggcaaga agttgattat 60
atgactcaga ctaggggtca gagatcctct ctggcagaag acaatgagtc cagctacagc 120
agaggattt acatgacgta cactgagtt gactatgact tatgcaatga agtggttgac 180
gtgacctgct cccctaagcc agatgcattc aacccatgtg aagatatcat ggggtacaac 240
atccctcagag tcctgatatg gtttatcagc atcctggcca tcactggaa catcatagtg 300
ctagtgatcc taactaccag ccaatataaa ctcacagtc ccaggttcct tatgtgcaac 360
ctggccttg ctgatctctg cattgaatc tacctgctgc tcattgcatt agttgatatac 420
cataccaaga gccaatatca caactatgcc attgactggc aaactggggc aggctgtgat 480
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accttggaaa gatggcatac catcacgcat gccatgcagc tggactgcaa ggtgcagtc 600
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6/7

Fig. 6

TCTCAGGAAGAAACTCATCATTCTACCCCTGCACAAAGACAG

A1

+855 TGATGTATTGCTATACTGGATCTGAGATGTTGATTCTATTCTTTGTATTTCTAGC

+856 TCTGAGCTTCATCCAATTGCAACAAATCTATTTAAGGCAAGAAGTTGATTATATGACT

EX-10 S E L H P I C N K S I L R Q E V D Y M T

+916 CAGACTAGGGGTCAAGAGATCCCTCTGGCAGAACATGAGTCCAGCTACAGCAGAGGA

Q T R G Q R S S L A E D N E S S Y S R G

B1 A2

976 TTTGACATGACGTACACTGAGTTGACTATGACTTATGCAATGAAGTGGTTGACGTGACC

F D M T Y T E F D Y D L C N E V V D V T

1036 TGCTCCCCTAAGCCAGATGCATTCAACCCATGTGAAGATATCATGGGGTACAACATCCTC

C S P K P D A F N P C E D I M G Y N I L

1096 AGAGTCCTGATATGGTTATCAGCATTGCCATCACTGGGAACATCATAGTGCTAGTG

R V L I W F I S I L A I T G N I I V L V

1156 ATCCTAACTACCAGCCAATATAAAACTCACAGTCCCCAGGTTCTTATGTGCAACCTGGCC

I L T T S Q Y K L T V P R F L M C N L A

C1 B2

1216 TTTGCTGATCTCTGCATTGGAATCTACCTGCTGCTATTGCATCAGTTGATATCCATACC

F A D L C I G I Y L L L I A S V D I H T

1276 AAGAGCCAATATCACAACTATGCCATTGACTGGCAAAC~~TGGGGCAGGCTGTGATGCTGCT~~

K S Q Y H N Y A I D W Q T G A G C D A A

1336 GGCTTTTCACTGTCTTGCCAGTGAGCTGTCAGTCTACACTCTGACAGCTATCACCTTG

G F F T V F A S E L S V Y T L T A I T L

D1

1396 GAAAGATGGCATACCACATCACGCATGCCATGCAGCTGGACTGCAAGGTGCAGCTCCGCCAT

E R W H T I T H A M Q L D C K V Q L R H

C2

1456 GCTGCCAGTGTCATGGTGATGGGCTGGATTTGCTTTGCAGCTGCCCTCTTCCCATC

A A S V M V M G W I F A F A A A L F P I

1516 TTTGGCCATCAGCAGCTACATGAAGGTGAGCATCTGCCCTGCCATGGATATTGACAGCCCT

F G I S S Y M K V S I C L P M D I D S P

E1

1576 TTGTCACAGCTGTATGTCATGTCCTCCTGTGCTCAATGTCTGGCCTTGTGGTCATC

L S Q L Y V M S L L V L N V L A F V V I

D2

1636 TGTGGCTGCTATATCCACATCTACCTCACAGTGCGGAAACCCCAACATCGTGTCCCTCT

C G C Y I H I Y L T V R N P N I V S S S

1696 AGTGACACCAGGATGCCAAGCGCATGGCCATGCTCATCTTCACTGACTTCCTGCATG

S D T R I A K R M A M L I F T D F L C M

F1 E2

1756 GCACCCATTTCTTCTTGCCTTGCCTCCCTCAAGGTGCCCCCTCATCACTGTGTCC

A P I S F F A I S A S L K V P L I T V S

7/7

Fig. 6 (continued)

1816 AAAGCAAAGATTCTGCTGGTTCTGTTCACCCCATCAACTCCTGTGCCAACCCCTTCCTC
K A K I L L V L F H P I N S C A N P F L
1876 TATGCCATCTTACCAAAAAACTTCGCAGAGATTCTTCATTCTGCTGAGCAAGTGTGGC
Y A I F T K N F R R D F F I L L S K C G
1936 TGCTATGAAATGCAAGCCCCAAATTATAGGACAGAAAACCTTCATCCACTGTCCACAACACC
C Y E M Q A Q I Y R T E T S S T V H N T
1996 CATCCAAGGAATGGCCACTGCTCTTCAGCTCCCAGAGTCACCAAGTGGTTCCACTTACATA
H P R N G H C S S A P R V T S G S T Y I
2056 CTTGTCCCTCTAAGTCATTTAGCCCCAAACTAAAACACAATGTGAAAATGTATCTGAGTA
L V P L S H L A Q N END
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G2
2166 CTACACATTTCATCTAATTAATATT

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NIESCHLAG, Eberhard
SIMONI, Manuela
BEHRE, Herrmann M
PEREZ, Maritza

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 Ser Glu Leu His
 20 cca att tgc aac aaa tct att tta agg caa gaa gtt gat tat atg act 163
 Pro Ile Cys Asn Lys Ser Ile Leu Arg Gln Glu Val Asp Tyr Met Thr
 5 10 15 20

25 cag act agg ggt cag aga tcc tct ctg gca gaa gac aat gag tcc agc 211
 Gln Thr Arg Gly Gln Arg Ser Ser Leu Ala Glu Asp Asn Glu Ser Ser
 25 30 35

30 tac agc aga gga ttt gac atg acg tac act gag ttt gac tat gac tta 259
 Tyr Ser Arg Gly Phe Asp Met Thr Tyr Thr Glu Phe Asp Tyr Asp Leu
 40 45 50

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 Cys Asn Glu Val Val Asp Val Thr Cys Ser Pro Lys Pro Asp Ala Phe
 55 60 65

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 85 90 95 100

50 atc cta act acc agc caa tat aaa ctc aca gtc ccc agg ttc ctt atg 451
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 105 110 115

55 tgc aac ctg gcc ttt gct gat ctc tgc att gga atc tac ctg ctg ctc 499
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 135 140 145

60 att gac tgg caa act ggg gca ggc tgt gat gct gct ggc ttt ttc act 595
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35 40 45

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30 50 55 60

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65 70 75 80

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180 185 190

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Ser Tyr Met Lys Val Ser Ile Cys Leu Pro Met Asp Ile Asp Ser Pro
225 230 235 240

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